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THEORETICAL PREDICTION OF THE UV ADSORPTION AND FLUORESCENCE SPECTRA OF TYROSINE AND PHENYLALANINE

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13. ABSTRACT *(Maximum 200 words)*

This report presents a theoretical study of the ground state and of the first excited state of phenylalanine and tyrosine to interpret the excitation absorption and the fluorescence emission spectra of the two molecules. The computations are performed with the Gaussian 90 Program Package using 3-21G basis sets. The ground state is computed with the HF option and the excited state with the Configuration Interaction, Single (CIS) Excitations option only. This report optimized the molecular geometries of both the ground and excited states and computed the excitation energies at both geometries. In conclusion, the excited state is due to a superposition of excitations from bonding orbitals in the alanine chain to a π orbital in the benzene ring and to an orbital in the C-C bond attached to the ring.

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PREFACE

The work described in this report was authorized under Contract No. DAAL03-91-C-0034. This work was started in May 1992 and completed in August 1992.

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1. INTRODUCTION

There are 23 amino acids that occur frequently in protein hydrolyzates. These are defined in Meister's book[1] as the common amino acids. Four of these, tryptophan, cystine, phenylalanine and tyrosine, exhibit characteristic ultraviolet absorption. Three of these amino acids, tryptophan, phenylalanine and tyrosine, reemit the absorbed ultraviolet light in the form of fluorescence. Measurements of the fluorescence spectra provide a useful method for the identification of those three amino acids and for the detection of proteins in general. In order to understand the dynamics and the mechanism of the fluorescence of these molecules it is helpful to study the assignment and the geometries of the various states involved in their fluorescence. In the present paper we present ab initio calculations on the ground states and the first excited singlet states of the two amino acids phenylalanine and tyrosine. These two molecules differ by one hydroxyl group only, and we shall see that there are only minor differences between the computed geometries and excitation energies of the two molecules. The tryptophan molecule is not discussed in the present paper. The theoretical approach that we use for the study of tyrosine and phenylalanine does not necessarily lead to reliable theoretical results for tryptophan.

The ultraviolet fluorescence spectra of the three amino acids tryptophan, phenylalanine and tyrosine were measured by Teale and Weber[2] in 1956. We realize that these experiments were performed some time ago, but the data seem quite reliable. They are the primary source of the fluorescence spectra of phenylalanine and tyrosine in most recent reviews, for example the book by Lakowicz[3].

The geometry of phenylalanine and tyrosine was the subject of a number of studies around 1970. It should be noted here that tyrosine is optically active and that there are slight differences in the crystal structures of D, L and DL-tyrosine. However, we found that the computed variations in bond lengths and bond angles between the various modifications are negligible in the isolated molecules. The unit cell dimensions and the space groups of tyrosine and phenylalanine were reported by Khawas[4,5] and by Khawas and Murti[6]. Mostad, Nissen and Roemming[7] presented a single crystal X-ray analysis of L-tyrosine. They found that their unit cell was half the size of the one reported by Khawas and Murti[6]. The bond distances and bond angles of L-tyrosine were derived from the X-ray measurements by Mostad, Nissen and Roemming[8]. In a subsequent paper the same data for DL-tyrosine were also reported by Mostad and Roemming[9].

Frey, Koetzle, Lehman and Hamilton[9] presented a precision neutron diffraction structure determination of L-tyrosine and of L-tyrosine hydrochloride. They also reviewed and evaluated all previous experimental data on the geometry parameters of tyrosine. We believe that the work reported by Frey et al represents the most accurate and reliable experimental information of the geometry parameters of

tyrosine, and we will use this work as a basis of comparison for our theoretical tyrosine data. It is unfortunate that experiments of similar accuracy are not available for phenylalanine. Comparisons between theoretical and experimental results are limited to one of the two molecules only. On the other hand, our computed bond distances and bond angles of the two molecules tyrosine and phenylalanine are practically identical, and we hope that our considerations for tyrosine are valid also for the other molecule. In the case of tyrosine we have to deal with another difficulty. Our computations refer to an isolated molecule and the theory predicts that the neutral molecule has the most stable configuration with the lowest energy. The neutron diffraction experiments were performed on crystalline compounds. One of the studied compound seemed to contain zwitterions and the other one positive ions. It is therefore awkward to make accurate comparisons between the computed and the experimental data.

2. COMPUTATIONS

In our computations on phenylalanine and tryptophan we used the Gaussian 90 Program package[10]. We used the Hartree Fock option combined with the 3-21G basis sets to compute the ground state energies and to determine the optimized ground state geometry parameters. We used the CIS option (configuration interaction, single excitations only) to compute the energy of the lowest excited state and its optimized geometry. All computations were performed on a Stardent 3000 VS computer.

2.1 Ground States.

We list the computed ground state bond distances of phenylalanine and tyrosine in Table 1. A visual representation of the computed ground state geometries is shown in Figures 1, 2, 3 and 4; Figures 1 and 2 show the phenylalanine molecule and Figures 3 and 4 show tyrosine. The numbering of the atoms is shown in Figures 1 and 3, which present a view perpendicular to the phenyl ring. The two molecules differ only by the presence of the phenyl group. We denote atom number 8 by the symbol Y. This represents a hydrogen atom in the case of phenylalanine and an oxygen atom in the case of tyrosine. The numbering of the other atoms is the same for the two molecules except that tyrosine has an additional atom, H24 attached to Y8 (O8). It may be seen in Table 1 that the bond distances in phenylalanine and tyrosine differ slightly in the vicinity of C2, where the one molecule has a hydroxyl instead of a hydrogen. In the amino acid parts the bond distances are practically identical in the two different molecules. All bond distances in Table I are in terms of angstroms. The accuracy of the computed bond distances is probably less that 0.001 angstroms, but we list the theoretical values to within four decimal places in order to show the small variations due to excitation and the small differences between tyrosine and phenylalanine.

We mentioned already that an accurate experimental structure determination of tyrosine was reported by Frey et al[9], but there are some problems in comparing the experimental results with our theoretical data. The computations on an isolated tyrosine molecule predict that the $C_6H_4(OH)CH_2CH(NH_2)COOH$ configuration has the lowest energy. The tyrosine single crystal consists of zwitterions with the

configuration $C_6H_4(OH)CH_2CH(NH_3)CO_2$. according to Frey et al[9]. There are significant differences between the geometries of the zwitterion and the neutral species. Frey et al[9] also report the experimental geometry of L-tyrosine hydrochloride, which contains the positive ion $C_6H_3O(NH_3)^+COOH$. We find that the structure of the positive ion is much more compatible with our computed structure of the neutral molecule than the zwitterion even though there are some discrepancies in the NH_3 group.

We list the experimental bond distances and bond angles of the tyrosine cation in Tables 1 and 2. The agreement between the experimental and the computed bond distances is satisfactory except for the C16-O20 bond length where the difference is 0.04 angstroms. It should be noted that the computed bond distances in tyrosine are almost identical with the corresponding bond distances in phenylalanine. Even in the vicinity of C2 there is very little difference.

The experimental bond angles of the cation[9] are listed in Table 2. Here the differences between the experimental and computed values are somewhat larger, but they are usually less than one degree. The largest discrepancy is found again in the carboxyl group: a difference of 2.1 degrees in the C13-C16-O20 angle. It should again be noted that the theoretical bond angles for phenylalanine are almost identical with those of tyrosine. The presence of the hydroxyl group seems to have but little effect on the molecular geometry.

We mentioned already that Figs. 1 and 3 present a view perpendicular to the phenyl ring of the two molecules. In order to get a better feel for the molecular geometries we present a different view in Figs. 2 and 4. We rotated the molecules around and decided that the positions of Figs. 2 and 4 show the positions of the alanine chains relative to the phenyl rings. We found that the CH_2COOH ring forms a plane parallel to the plane of the phenyl ring with the nitrogen sticking out a bit. The carboxyl hydrogen H21 points towards the nitrogen. The N17-H21 distance is 1.917 angstroms in phenylalanine and it is 1.919 angstroms in tyrosine. Our computations show that the hydrogen prefers attachment to the carboxyl group and that the zwitterion has a higher energy than the neutral configuration. The computations refer to the isolated molecule. It is possible that in a crystal or in an aqueous environment the conclusion might be different.

2.2 Excited States.

In order to study the first excited state of phenylalanine and tyrosine we performed a configuration interaction (singles) calculation including the first 5 excited states(the CIS option). Again we used the 3-21G basis set. We derived the optimized geometries of the lowest excited states of the two molecules. We present the computed bond distances of the excited states in Table 1 and the computed bond angles in Table 2.

The changes in geometry between the ground and first excited states are fairly small in both molecules and we were unable to detect them visually. Therefor, figs 1-4 also depict the geometries of the excited states of the two molecules. An inspection of Tables 1 and 2 reveals that the most significant changes in geometry occur in the carboxyl groups. The C=O bond lengths increase by almost 0.1

angstroms and the C-O bond lengths increase by 0.06 angstroms. Similarly, the only significant change in bond angle occurs in the C13-C16-O19 bond angle. A slightly smaller change occurs in the C13-C16-O20 bond angle. The geometry of the phenyl ring remains practically the same. We may therefore conclude that the major changes in geometry occur in the carboxyl groups.

In order to obtain a better understanding of the electronic rearrangements due to molecular excitation we computed the Mulliken atomic charge densities of the two molecules in the ground and excited states, the values are reported in Table 3. It should be noted that the excited state Mulliken densities refer to the excited state optimized geometry. It may be seen in Table 3 that the most dramatic changes in atomic density are to be found in C16 and in O19, that is in the C=O bond. We find that O19 loses 0.55 of an electron and that C16 gains 0.66 of an electron upon excitation. Roughly speaking, about half an electronic charge moves along the C=O bond from the oxygen to the carbon atom upon excitation. There are also some changes in charge density in the other carboxyl oxygen and in C13, the carbon bonded to the carboxyl group. However, these changes are of the order of 0.05 electron only.

The excited state may also be interpretated from an analysis of the molecular orbitals involved and this interpretation seems to be different from the above. In the case of phenylalanine the lowest excited state is a superposition of the excitations 40 –> 45, 40 –> 47, 42 –> 45 and 42 –> 47. Molecular orbital 40 is a delocalized bonding orbital. It extends from N17 via C13 and C16 to O19 and O20. That is to say it extends throughout the whole alanine chain. Orbital 42 is similar to orbital 40. It also extends through the area N17-C13-C16-O19,O20. Orbital 45 is a delocalized π orbital in the phenyl ring and orbital 47 is a localized C1-C7 orbital. The analysis of the molecular orbitals leads therefore to the conclusion that the lowest excited state involves the transfer of an electron from the alanine chain to the phenyl ring and to the adjacent C1-C7 bond.

It may be said that an analysis of the molecular orbitals and an analysis of the Mulliken charge densities are not entirely consistent with one another. We believe that the conclusions derived from the molecular orbital analysis are more reliable. Our conclusion is therefore that the molecular excitation involves both the benzene ring and the alanine chain. This conclusion is equally valid for both phenylalanine and tyrosine.

We performed similar computations on alanine, phenol, toluene and o-cresole. The lowest excitation energy in all these molecules are quite a bit higher than in phenylalanine and tyrosine. This supports our conclusion that in the latter two molecules the lowest excited state involves an electron transfer between the amino acid chain and the ring. We cannot explain why this electron transfer is not shown more explicitly by the Mulliken atomic charges of Table 3.

The most important theoretical results for the description of the fluorescence are the molecular excitation energies. We list the various computed energies for phenylalanine and tyrosine in Table 4. In the first line we list the total molecular energies at the optimized molecular geometries, corresponding to the energy minima

of the molecules. In the second line we list the molecular energies at the molecular geometries where the first excited states have energy minima. The energies at the first two lines of Table 4 are expressed in terms of hartrees. The energy differences between the molecular ground states and the lowest excited states (both at the same ground state optimized geometries) are given on the third line and are expressed in terms of eV. The corresponding excitation wavelengths in terms of nm are on the fourth line.

We mentioned already that we minimized the energy of the lowest excited state with respect to the molecular geometry. The corresponding geometry parameters are presented in Table 1 and 2 and the corresponding excitation energy (at the excited state optimized geometry) is presented on the seventh line of Table 4 (in terms of eV). The corresponding wavelengths in terms of nm are listed on the last line of the Table. The latter wavelengths correspond to the molecular fluorescence emission.

2.3 Molecular Vibrations.

So far we have not considered the effects of molecular vibrations on the molecular excitation and fluorescence emission spectra. In order to determine those effects we computed the vibrational frequencies of the two molecules in their ground states. The sums of the zero-point vibrational energies in terms of eV are presented on the fifth line of Table 4. If we correct the excitation energies for the effects of those zero-point vibrational energies then we predict the corrected excitation wave lengths of the sixth line of the table. We should introduce a similar correction on the fluorescence wave lengths but we were unable to compute the excited state vibrational frequencies with our present computer capability. The excited state vibrational frequencies are always smaller than the corresponding ground state frequencies but we do not know how much smaller. If we had to make a guess we would predict that the zero-point vibrational correction would cause an increase of 10 to 15% in the fluorescence wavelengths but this is only a very rough guess since there is no quantitative information to support it.

3. DISCUSSION

The available experimental data on the excitation absorption spectra and the fluorescence emission spectra of phenylalanine and tyrosine are reviewed in the recent book by Lakowicz[3]. The excitation spectra and the fluorescence emission spectra of phenylalanine and tyrosine in aqueous solution were measured as early as 1956 by Teale and Weber[2]. More recent studies of the excitation and fluorescence spectra are concerned with features such as polarization, quenching, energy transfer between different molecules, etc. but the spectral frequency distributions of UV excitation and fluorescence are always attributed to Teale and Weber[2].

The final comparison between our computed peaks of the excitation absorption and the fluorescence emission spectra and the corresponding experimental values as reported by Teale and Weber[2] is presented in Table 5. It should be noted that we have corrected the absorption peaks for the effects of zero-point vibrations in the electronic ground state. We did not correct our computed fluorescence peak values

because we do not know the vibrational frequencies of the excited states. It is generally known that the vibrational frequencies of higher electronic states are always smaller than the corresponding values of the electronic ground state. In a typical case for a diatomic molecule the ratio is less than one half. If we use a similar ratio as the basis for a very rough guess then the theoretical fluorescence wavelengths of Table 5 should be adjusted downwards by about 15%.

The agreement between the experimental and the computed values of Table 5 seems to be satisfactory. It should be noted that the experiments were conducted in aqueous solution whereas the computations were performed on an isolated single molecule. There is every indication that the amino acids become zwitterions in aqueous solution so that the computations and the experiments refer to different modifications of the molecules. The experimental wavelengths may also be shifted due to interactions with solvent molecules. Finally, there is the effect of molecule vibrations. We have tried to account for the effect of molecular vibrations in the molecular ground state but we were unable to account for the effects of molecular vibrations in the molecular excited state. The latter may cause corrections of both the excitation and the fluorescent wavelengths. We believe that all uncertainties in both the experimental and the theoretical values can easily cause differences of as much as 20%. Therefore we believe that the agreement between our computations and the available experimental information should be considered satisfactory.

4. CONCLUSIONS

We briefly summarize the main conclusions of our computations on phenylalanine and tyrosine.

The theoretical results of phenylalanine and tyrosine are practically identical, the introduction of the hydroxyl group in tyrosine seems to have a very limited and a very localized effect on the molecular geometry parameters. The excitation and fluorescence wavelengths of the two molecules are practically identical. It seems therefore that the experimental differences between the two molecules are not accounted for by our computations.

The lowest excited state of phenylalanine is a superposition of the excitations 40 –> 45, 40 –> 47, 42 –> 45 and 42 –> 47. Molecular orbitals 40 and 42 are localized in the alanine chain, molecular orbital 45 is a delocalized π orbital in the benzene ring and orbital 47 is located in the C1-C7 bond adjacent to the ring. We conclude therefore that the lowest excited state involves the transfer of an electron from the alanine chain to the benzene or phenol ring.

It should be noted that the changes in molecular geometry of Tables 1 and 2 and the Mulliken atomic charge densities do not support this conclusion. The major changes in molecular geometry due to excitation are concentrated in the C=O and C-O bond distances and in the C-C-O bond angle. The major change in Mulliken charge density is a charge transfer of around 0.6 electron in the C=O bond. On the other hand, we performed similar computations on alanine, toluene and o-cresole separately and found that the first excitation in those molecules were significantly higher than in tyrosine and phenylalanine. The latter result seems to support the

idea that the excitation in tyrosine and phenylalanine involves both the ring and the alanine chain.

We found that the lowest excited state in phenylalanine and in tyrosine is non-degenerate, the next higher excited state is about 2 eV higher. This means that the CIS option we used in our computations should lead to reliable conclusions. The lowest excited state of tryptophan is near-degenerate, in that case the use of multiconfiguration SCF methods is required in order to derive reliable theoretical results. It seemed therefore advisable to postpone a detailed study of tryptophan until a later time.

Table 1. Bond Distances of Phenylalanine and Tyrosine

Bond	Phenylalanine		Tyrosine		
	Gr. st.	Exc. st.	Gr. State	Exc. St.	Exp[9]
C1-C3	1.3870	1.3881	1.3897	1.3909	1.393
C1-C4	1.3900	1.3901	1.3864	1.3864	1.393
C1-C7	1.5150	1.5146	1.5145	1.5142	1.509
C2-C5	1.3815	1.3823	1.3824	1.3830	1.393
C2-C6	1.3862	1.3856	1.3831	1.3824	1.382
C2-Y8	1.0717	1.0717	1.3746	1.3754	1.374
C3-C5	1.3864	1.3858	1.3803	1.3798	1.391
C3-H9	1.0736	1.0733	1.0734	1.0731	1.391
C4-C6	1.3815	1.3821	1.3820	1.3828	1.394
C4-H10	1.0733	1.0734	1.0732	1.0732	1.074
C5-H11	1.0720	1.0721	1.0695	1.0696	1.086
C6-H12	1.0720	1.0721	1.0729	1.0730	1.091
C7-C13	1.5494	1.5494	1.5499	1.5493	1.536
C7-H14	1.0823	1.0826	1.0824	1.0827	1.080
C7-H15	1.0840	1.0818	1.0842	1.0820	1.085
C13-C16	1.5297	1.5457	1.5291	1.5457	1.520
C13-N17	1.4712	1.4680	1.4708	1.4679	1.492
C13-H18	1.0819	1.0814	1.0821	1.0817	1.100
C16-O19	1.2019	1.2992	1.2019	1.2993	1.205
C16-O20	1.3388	1.4059	1.3390	1.4060	1.304
N17-H22	1.0063	1.0054	1.0063	1.0055	1.017
N17-H23	1.0047	1.0044	1.0046	1.0044	1.007
O20-H21	0.9782	0.9781	0.9780	0.9780	-
O8-H24	-	-	0.9642	0.9642	0.988

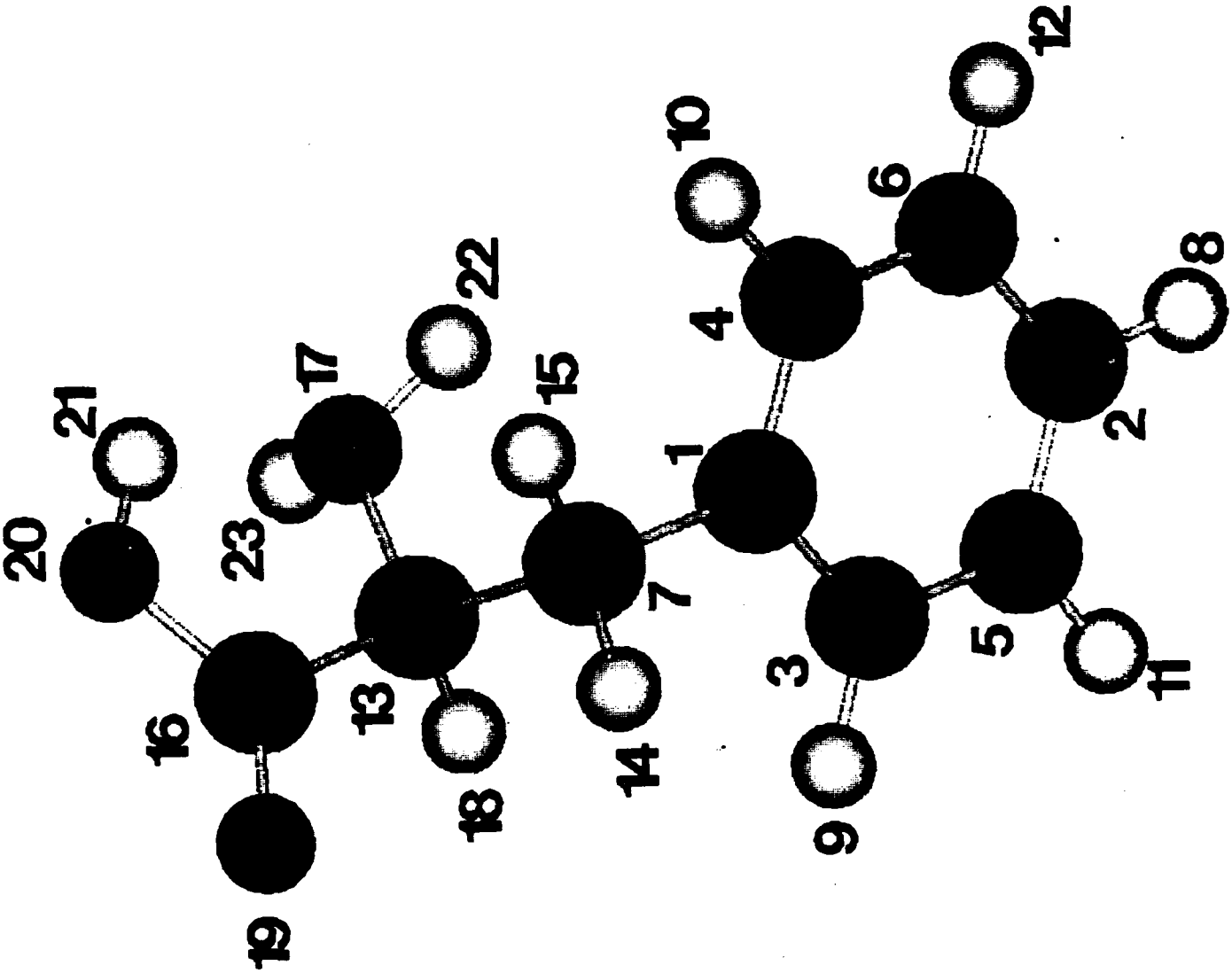

Figure 1. Optimized Ground State Geometry of Phenylalanine

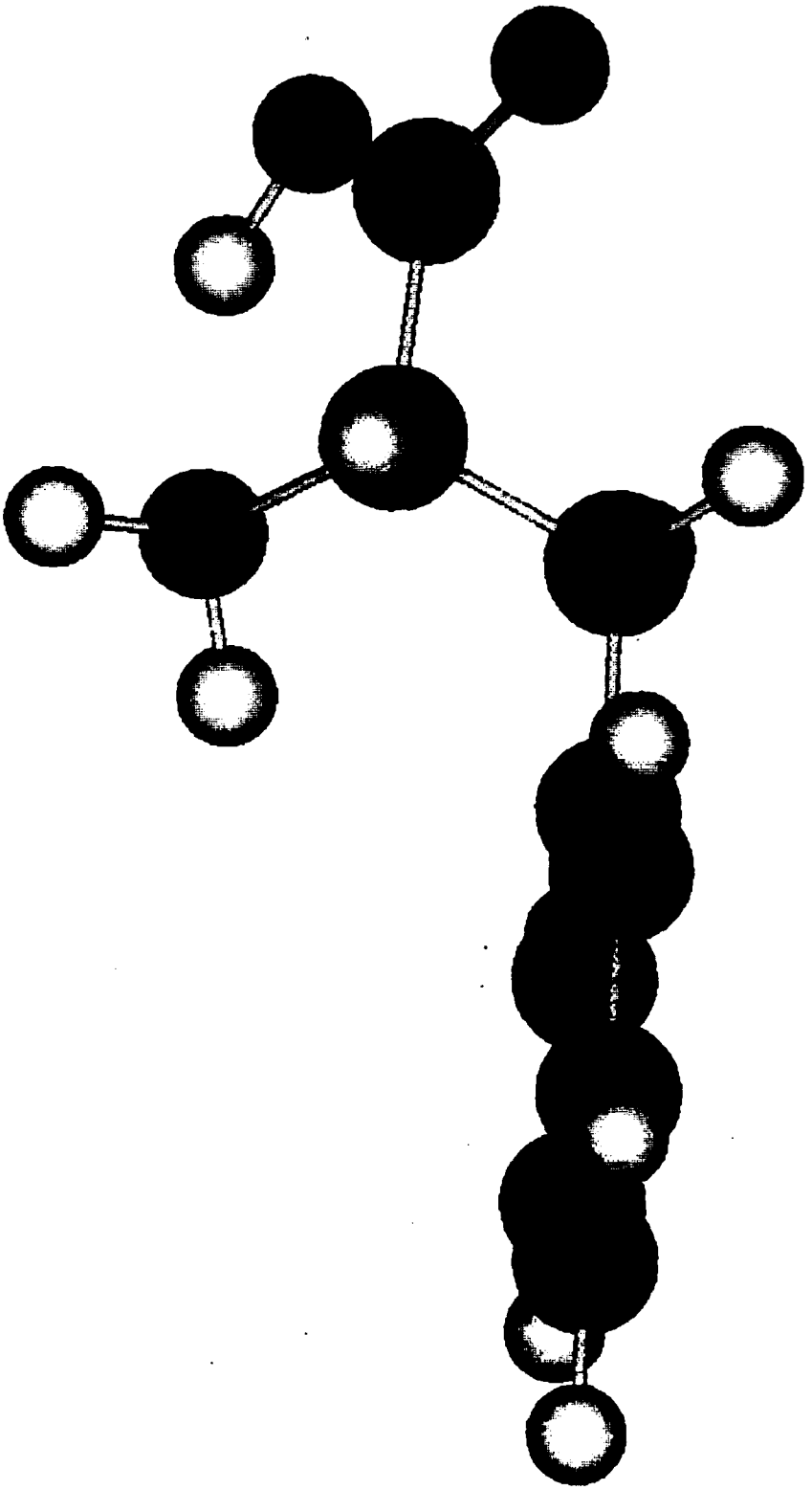

Figure 2. Side View of Phenylalanine Geometry

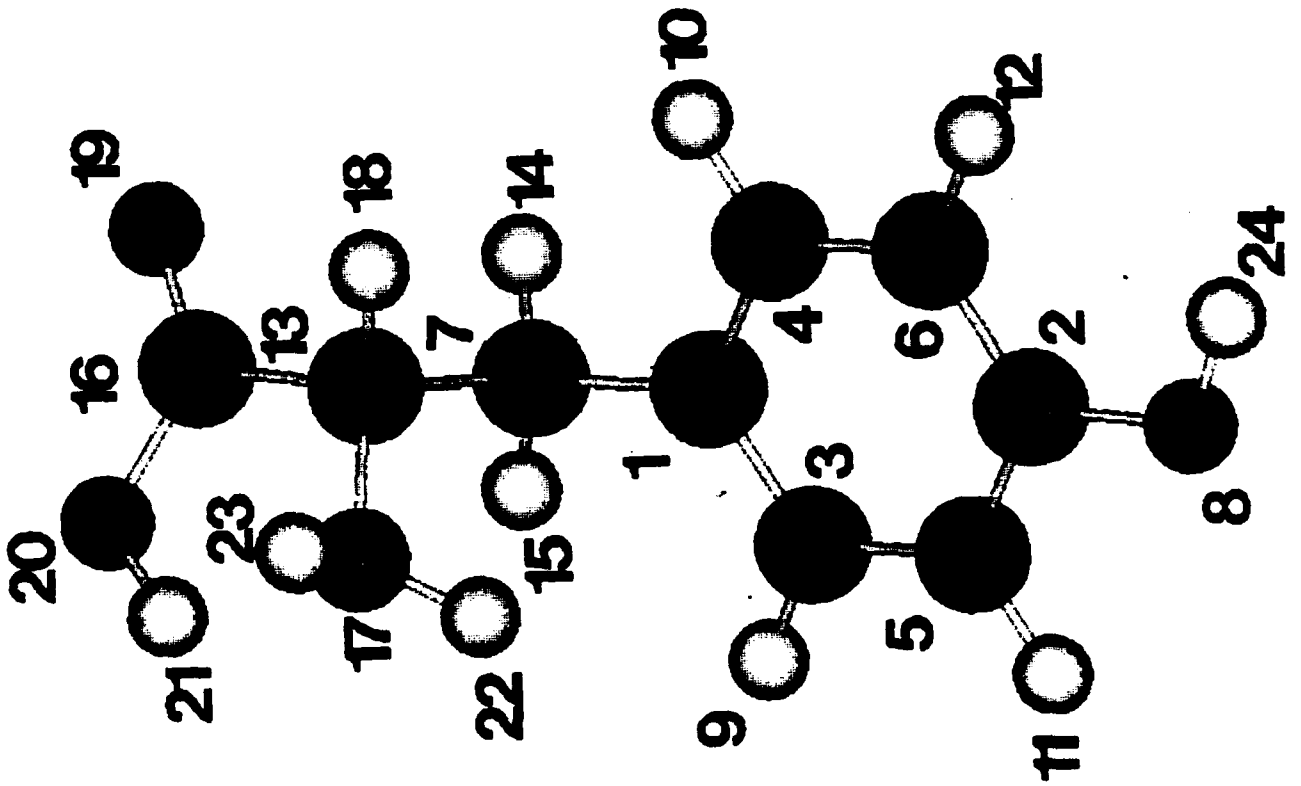

Figure 3. Optimized Ground State Geometry of Tyrosine

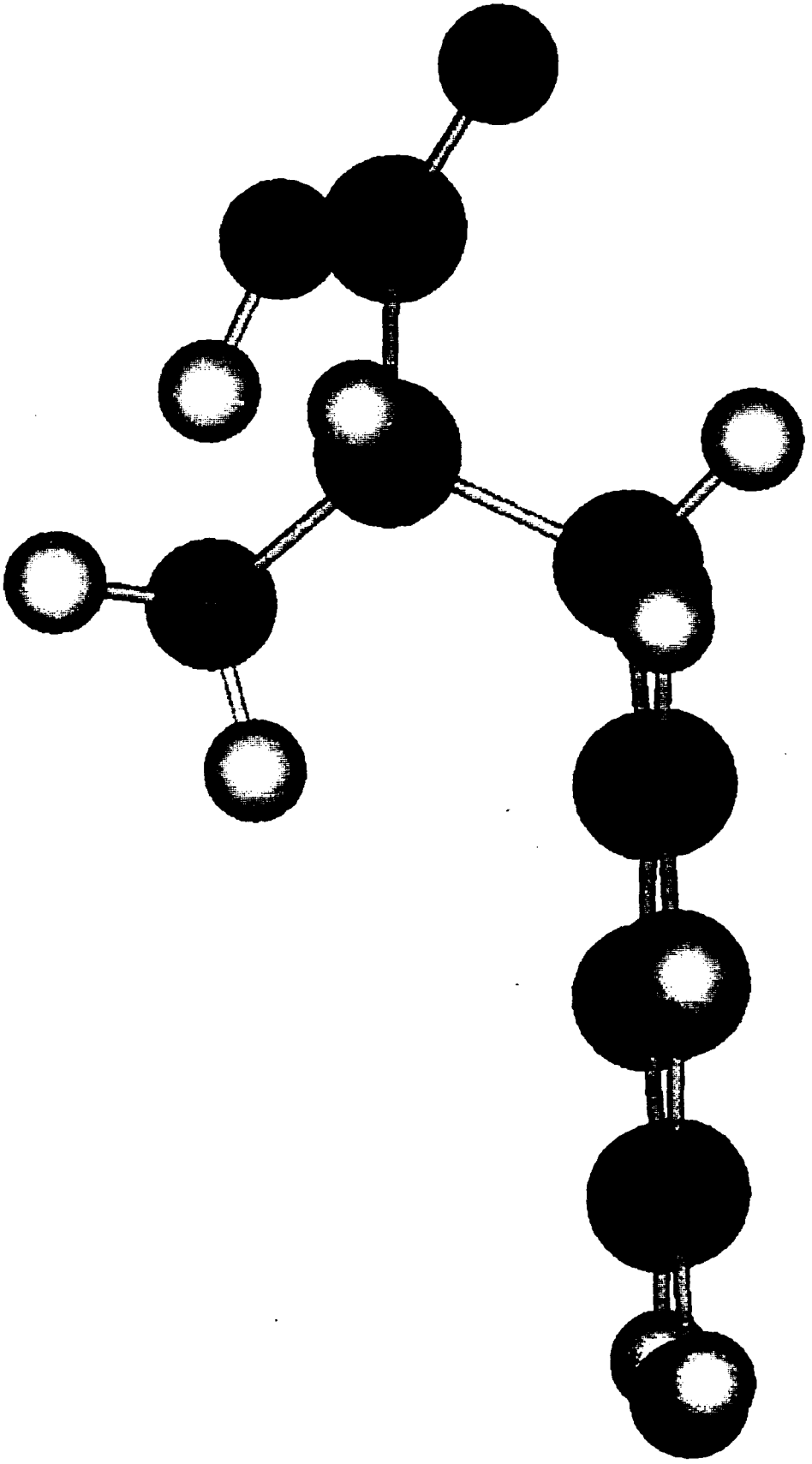

Figure 4. Side View of Tyrosine Geometry

Table 2. Bond Angles of Phenylalanine and Tyrosine

Bond Angle	Phenylalanine		Tyrosine		
	Gr. st.	Exc. st.	Gr. St.	Exc. St.	Exp[9]
C3-C1-C4	118.79	118.67	118.04	117.93	118.5
C3-C1-C7	120.87	120.93	121.25	121.20	121.2
C4-C1-C7	120.33	120.38	120.70	120.85	120.2
C5-C2-C6	119.62	119.59	119.47	119.45	120.2
C5-C2-Y8	120.15	120.24	117.30	117.30	117.3
C6-C2-Y8	120.15	120.17	123.23	123.25	122.5
C1-C3-C5	120.68	120.71	121.23	121.27	120.8
C1-C3-H9	119.65	119.52	119.63	119.49	120.1
C1-C4-C6	120.63	120.72	121.08	121.15	121.0
C1-C4-H10	119.61	119.56	119.73	119.69	119.2
C2-C5-C3	120.09	120.14	120.03	120.05	119.7
C2-C5-H11	120.13	120.09	118.52	118.50	119.7
C2-C6-C4	120.19	120.18	120.16	120.15	119.6
C2-C6-H12	119.98	119.99	120.16	120.16	120.1
C1-C7-C13	111.99	112.37	112.21	112.59	113.5
C1-C7-H14	110.93	110.41	111.02	110.52	109.8
C1-C7-H15	110.20	110.91	110.15	110.82	-
C7-C13-C16	107.12	109.34	107.15	109.38	109.2
C7-C13-N17	110.75	109.45	110.66	109.45	110.9
C7-C13-H18	108.71	110.08	108.72	110.09	110.8
C13-C16-O19	122.40	115.79	122.43	115.77	122.0
C13-C16-O20	113.76	110.72	113.74	110.71	111.6
C13-N17-H22	113.29	113.24	113.31	113.27	113.3
C13-N17-H23	113.07	113.09	113.03	113.04	-
C16-O20-H21	108.41	103.93	108.46	103.95	110.7
C2-O8-H24	-	-	113.22	113.14	113.0

Table 3. Mulliken Atomic Charge Densities of Phenylalanine and Tyrosine

	Phenylalanine		Tyrosine	
Atom	Gr. St.	Exc. St.	Gr. St.	Exc.St.
C1	-0.063902	-0.082186	-0.085409	-0.102979
C2	-0.244135	-0.243842	0.376844	0.376811
C3	-0.251674	-0.247489	-0.235473	-0.231285
C4	-0.227360	-0.225448	-0.211363	-0.209744
C5	-0.229008	-0.230540	-0.244443	-0.245849
C6	-0.228580	-0.230032	-0.274400	-0.276320
C7	-0.424461	-0.418335	-0.421502	-0.414663
Y8	0.246388	0.246639	-0.744869	-0.744130
H9	0.246564	0.244686	0.246270	0.250395
H10	0.244935	0.245226	0.250191	0.250395
H11	0.246564	0.246790	0.270004	0.270295
H12	0.247494	0.247322	0.240365	0.240151
C13	-0.178952	-0.126036	-0.172906	-0.125552
H14	0.273380	0.271127	0.270277	0.268956
H15	0.241335	0.252961	0.240647	0.252461
C16	0.926556	0.264481	0.922102	0.263805
N17	-0.837159	-0.816153	-0.835552	-0.815842
H18	0.259910	0.249870	0.255222	0.247261
O19	-0.612590	-0.059342	-0.611666	-0.059821
O20	-0.736159	-0.679850	-0.734831	-0.679739
H21	0.441987	0.427156	0.441229	0.427178
H22	0.322351	0.316987	0.321523	0.316702
H23	0.342610	0.346007	0.341894	0.345354
H24	-	-	0.395846	0.395914

Table 4. Computed Energies and Excitation Energies

	Phenylalanine	Tyrosine
Ground St. En.(Gr. St. Geom.)	-548.336 857	-622.777 015
Ground St En.(Exc. St. Geom.)	-548.285 225	-622.725 421
Excitation En.(Gr. St. Geom.)	6.2767 eV	6.2594 eV
Exc. wavelength	197.53 nm	198.07 nm
Zero-point vibr.	1.3301 eV	1.3358 eV
Corr. Exc. wavel.	250.65 nm	251.82 nm
Excitation En.(Exc. St. Geom.)	4.0842 eV	4.0868 eV
Fluor. wavelength	303.57 nm	303.37 nm

Table 5. Comparison Between Computed and Experimental[2] Peaks

wavelength	Phenylalanine	Tyrosine
excit. exp.	258 nm	274 nm
excit theor.	251 nm	252 nm
fluor. exp.	282 nm	303 nm
fluor. theor.	304 nm	303 nm

Blank

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